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TAURINE UPTAKE IN SARCOLEMMA VESICLES ISOLATED FROM HYPERTROPHIC GUINEA-PIG HEARTS

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Abstract—Taurine uptake by guinea-pig hypertrophic heart sarcolemma vesicles was sodium-dependent and kinetic analysis suggested the presence of only one uptake system. The apparent K_m and V_{max} were the following; 2.5×10^{-6} M and 0.43 pmoles·mg^{-1} protein·min^{-1} respectively. The uptake was specific and was inhibited by all analogues tested, except isethionic acid. The uptake was also inhibited by NaF.

The aminoacid taurine occurs in high concentrations in many animal tissues [1]. The mammalian heart contains high concentrations [1] but its function is still unknown although it seems to modulate ionic fluxes and stabilizes subcellular membranes responsible for calcium transport during excitation–contraction coupling [2].

Taurine tissue levels are very stable and, in fact, increased taurine concentrations have been found only in advanced pathophysiological states [3–5]. It seems that, at least in the rat heart, taurine transport is fundamental to the maintenance of the tissue levels [5] and it is regulated by β-adrenergic receptors [6].

The taurine uptake into guinea-pig sarcolemma isolated from normal hearts is sodium and temperature-dependent and two uptake sites are present. The transport is inhibited by β-alanine, hypotaurine homotaurine and guanidoethylsulphonate while isethionic acid increases it [7].

We now report our findings on taurine transport into guinea-pig sarcolemma vesicles isolated from hypertrophic hearts.

METHODS

Cardiac hypertrophy in guinea-pig was induced by s.c. injection of isoprotenerol at a dose of 2.5 mg/kg at 12 hr intervals for 10 days [8]. After this treatment the animals were killed and the heart was washed to remove the blood and placed in ice cold 0.25 M sucrose. The membranes were prepared from ventricles as described by Kidwai *et al.* [9], using a sucrose gradient. The sarcolemma obtained by this method was in the form of vesicles [9]. The purity of membranes was examined by determining the activities of several enzyme markers. Na^+–K^+ ATPase activity was measured according to Sulakhe and Dhalla [10], succinate dehydrogenase according to King [11] and glucose-6-phosphatase as described by Kidwai *et al.* [9]. The activities of these enzymes in the sarcolemma preparations were the following: Na^+–K^+ ATPase 7 Pi μmoles·mg^{-1} protein·min^{-1}, glucose-6-phosphatase 0.28 Pi μmoles·mg^{-1} protein·min^{-1} and succinate dehydrogenase 0.28 μmoles·succinate·mg^{-1} protein·min^{-1}. The results are in agreement with those of other authors according to which the membranes are in a satisfactory degree of purity.

The membranes were suspended in Tris maleate buffer 10 mM, pH 7, containing 1.2 mM $MgSO_4$, 4.8 mM KCl, 1.25 mM $CaCl_2$ and 120 mM NaCl and were stirred for 30 min at 0°C to obtain a homogeneous suspension. The incubation mixture (120 μl) contained 40–50 μg of proteins and 60 pmoles of [^{3}H]taurine. In some experiments the membranes were disrupted by hypo-osmotic shock in water and then centrifuged and resuspended in the standard incubation buffer. The incubation mixtures were shaken for 30 min at 20°. In some experiments NaCl was substituted with choline chloride to study whether taurine uptake is Na^+ dependent; after 30 min of incubation the membranes were mixed with 2.5 ml of ice cold incubation buffer and then filtered on Whatman GF/C filters. The filters were then quickly washed by vacuum filtration with 3 ml of ice cold buffer three times. The entire filtration procedure took less than 20 sec. After drying, filters were placed in triton/toluene [12] and then counted in a Packard liquid scintillation spectrometer (Packard Instruments).

Separate incubations were carried out in each assay in the presence of a high concentration (0.7 M) of unlabelled taurine to assess unspecific uptake. Data from competitive experiments were graphically analyzed with Lineweaver-Burk plot. Membrane protein concentrations were determined using the method of Weichselbaum [13]. [^{3}H]Taurine (sp. act. 20.5 Ci/μmole) was purchased from the Radiochemical Centre, Amersham.

RESULTS

The uptake of taurine in the complete absence of sodium was reduced to less than 10 per cent of that in the normal medium. Increasing Na^+ concentrations in the incubation medium increased the uptake of taurine as shown in Table 1. The time course of [^{3}H]taurine transport is shown in Fig. 1. It was linear

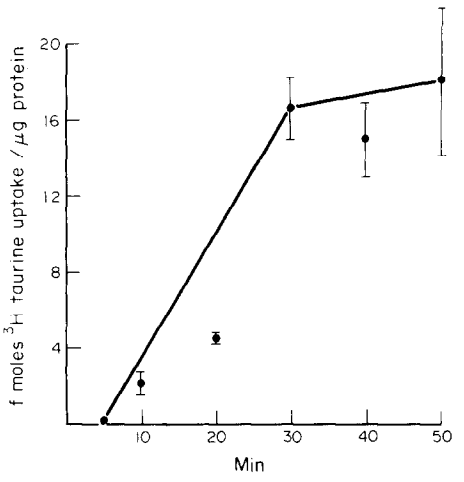

Fig. 1. Time course of [^{3}H]taurine uptake. The results are the means ± S.E. of 5 experiments performed in triplicate.

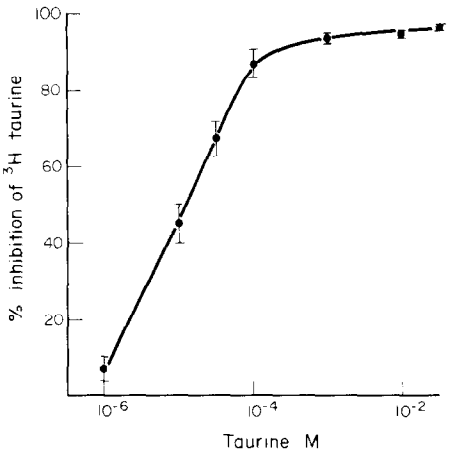

Fig. 2. Competition curve of [^{3}H]taurine by cold taurine. Results are expressed as means ± S.E. of 7 experiments performed in triplicate.

Table 1. Effect of metabolic inhibitors, Na^+ concentrations and hypo-osmotic shock on the uptake of [^{3}H]taurine in myocardial membrane vesicles

Inhibitors	Taurine (% of control)
None	100
NaF 10^{-3} M	56.21 ± 11.24 (4)*
NaCN 10^{-4} M	83.86 ± 3.70 (4)
Without Na^+	9.25 ± 0.81 (5)†
Na^+ 60 mM	39.01 ± 1.14 (5)†
Hypo-osmotic shock	5.12 ± 0.97 (4)†

The results (mean ± S.E.) are given as percentage of the control values. Difference from the control values *$P < 0.05$, †$P < 0.001$. Number of experiments in brackets.

for the first 30 min rising to 14.0 fmole · μg^{-1} protein and was markedly reduced when the membranes were submitted to hypo-osmotic shock (Table 1). Competition by cold taurine for [^{3}H]taurine uptake is shown in Fig. 2. The Lineweaver–Burk plot of taurine transport was found to fit a straight line equation ($r = 0.99$) (Fig. 3), thus making its kinetics conform to a high affinity process with an apparent K_m of 2.5×10^{-6} M and V_{max} of 0.43 pmoles · mg^{-1} protein · min^{-1}. Taurine transport was inhibited by NaF (an inhibitor of ATPase) [13] while NaCN did not reduce it significantly (Table 1). The structural analogues of taurine inhibited the uptake with the exception of isethionic acid (Table 2).

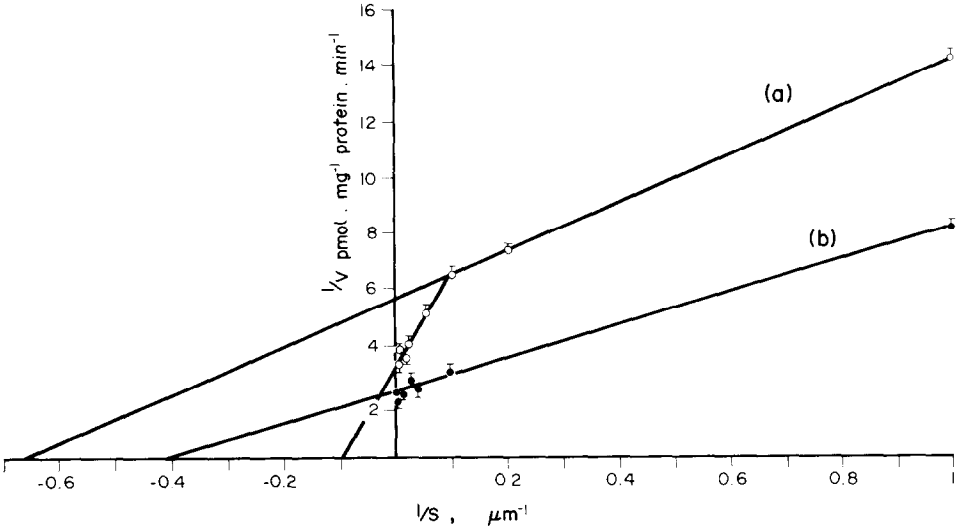

Fig. 3. Lineweaver–Burk plot of competition curve of [^{3}H]taurine uptake by cold taurine into plasma membranes isolated from normal (a) and hypertrophic (b) hearts. The data utilized in plot (a) were obtained by Franconi *et al.* [7]. The normal heart presents two uptake systems: the low affinity one with a K_m of 15.00 μM and V_{max} of 30 pmoles · mg^{-1} protein · min^{-1}; the high affinity one with a K_m of 1.94 μM and a V_{max} of 0.18 pmoles · mg^{-1} protein · min^{-1}. The hypertrophic heart presents a K_m of 2.5 μM and a V_{max} of 0.43 pmoles · mg^{-1} protein · min^{-1}. Each point is mean ± S.E. of at least 5 experiments. The lines are fitted by linear regression analysis.

Table 2. Effect of structural analogues of taurine on the uptake of ^3H taurine into myocardial membrane vesicles

Structural analogues	fmoles/μg protein
None	14.86 ± 1.75 (16)
β alanine	
10 mM	1.71 ± 0.5 (5)
1 mM	1.96 ± 0.45 (5)
Homotaurine	
10 mM	1.79 ± 0.21 (5)
1 mM	4.40 ± 0.85 (5)
Hypotaurine	
1 mM	1.63 ± 0.65 (3)
Guanidoethylsulphonate	
1 mM	3.52 ± 1.02 (3)
Isethionic acid	
10 mM	14.6 ± 2.02 (5)
1 mM	14.03 ± 1.46 (5)

The results are mean ± S.E. The number of experiments in brackets.

DISCUSSION

The transport mechanism of taurine into the sarcolemma isolated from hypertrophic heart is sodium dependent and slow as shown in Fig. 1. This transport is specific; in fact it is inhibited by β-alanine, homotaurine, hypotaurine and guanidoethylsulphonate. Azari *et al.* [14] also demonstrate that the above compounds inhibit taurine uptake in rat hearts. The compounds that inhibit the taurine transport present two or three carbon atoms between the acid and basic groups of the molecule and the presence of the aminic group is also necessary; in fact isethionic acid does not inhibit the uptake. The same results are also obtained in rat brain slices [15].

The isethionic acid exerts a different action on the taurine uptake into sarcolemma vesicles isolated from normal heart; in fact in this case this organic acid increases the uptake in a statistically significant manner [7].

The different actions of NaF and NaCN could depend on the fact that the former also inhibits the Na–K ATPase [13].

The kinetic analysis of the inhibition curve (Fig. 3) demonstrates the presence of one uptake system with a K_m of 2.5 μM and V_{max} 0.43 pmoles · mg^{-1} protein · min^{-1}. The kinetics of taurine transport into sarcolemma preparations obtained from hypertrophic heart are different from those observed in the sarcolemma isolated from normal heart [7]. In fact, as shown in Fig. 3, in the normal heart two uptake systems are present.

It is interesting to point out that in the hypertrophic heart the low affinity transport site has disappeared; while the double reciprocal plots of the inhibition curve of taurine in normal and hypertrophic heart for the high affinity transport are essentially parallel. The affinity decreases from 1.94 to 2.5 μM while the V_{max} has increased. These data suggest that the rate of translocation of taurine from the transport site into the inside of vesicles increases. Our findings agree with Chubb and Huxtable [5] in rat heart and also support the idea that in guinea-pig hypertrophic heart there is a modification of taurine uptake.

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